

## Amendments to the Claims

65. (Currently Amended) A method for detecting a target nucleic acid, the method comprising the steps of:

separating a sample in which a target nucleic acid is present in an amount less than about 20% relative to non-target nucleic acid in said sample, ~~thereby~~ to form a plurality of assay samples;

~~exposing said assay samples to an amplification reaction designed to amplify~~  
C1 amplifying said target nucleic acid in said assay samples;

hybridizing the amplification product to a first molecular beacon probe which hybridizes to the target nucleic acid and to a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the first molecular beacon probe further comprises a first stem which comprises about 4 base pairs having a sequence 5'-CACG-3', and a first loop structure which comprises about 16 base pairs and has a  $T_m$  of about 50-51°C; and

detecting said target nucleic acid as the presence of the amplification product the first and the second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

66. (Currently Amended) A method for detecting a target nucleic acid, the method comprising the steps of:

providing a sample comprising X% of a target nucleic acid, wherein X is less than

100;

dividing the sample to produce a plurality of assay samples;

wherein the ratio of target to non-target nucleic acid in at least one of the samples is greater than X%;

amplifying the ~~single~~ target nucleic acid to form an amplification product;

hybridizing the amplification product to a first molecular beacon probe which hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the first molecular beacon probe further comprises a first stem which comprises about 4 base pairs having a sequence 5'-CACG-3', and a first loop structure which comprises about 16 base pairs and has a T<sub>m</sub> of about 50-51°C; and

detecting the ~~target nucleic acid as the presence of the amplification product~~ first and second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

67. (Currently Amended) A method for detecting a target nucleic acid in a population of non-target nucleic acid contained in a sample, the method comprising:

dividing a heterogenous sample comprising target nucleic acid and non-target nucleic acid to form a plurality of assay samples, wherein the concentration of non-target nucleic acid is at least ~~5-fold~~ 5-fold that of target nucleic acid in the heterogeneous sample, and wherein at least one of the assay samples comprises a single molecule of the target nucleic acid molecule;

amplifying the single molecule of target nucleic acid to form an amplification product;

hybridizing the amplification product to a first molecular beacon probe which hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the first molecular beacon probe further comprises a first stem which comprises about 4 base pairs having a sequence 5'-CACG-3', and a first loop structure which comprises about 16 base pairs and has a  $T_m$  of about 50-51°C; and

detecting the ~~amplification product as indicative of the presence in the sample of the target nucleic acid~~ first and the second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

68. (Currently Amended) The method of claim 66 or 111, wherein X is less than 20.

69. (Canceled)

70. (Currently Amended) The method of claim 65, 66, ~~or 67~~, 110, 111, or 112, wherein the target nucleic acid is a mutant nucleic acid.

71. (Currently Amended) The method of claim 65, ~~or 67~~, 110, or 112, wherein the non-target nucleic acid is a wild-type nucleic acid.

72. (Currently Amended) The method of claim ~~65, 66 or 67~~, 102 wherein the sample is diluted so that between about 0.1 and about 0.9 of the assay samples yield an amplification product when amplified.

73. (Original) The method of claim 72, wherein the sample is diluted so that between about 0.1 and about 0.6 of the assay samples yield an amplification product when amplified.
74. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein one out of every two assay samples contains on average one target nucleic acid.
75. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein the target nucleic acid is a genomic DNA.
76. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112 wherein the target nucleic acid is a cDNA.
77. (Original) The method of claim 76, wherein the cDNA is generated by RT-PCR of at least one RNA.
78. (Currently Amended) The method of claim 65 or 110, wherein at least ten assay samples are analyzed.
79. (Original) The method of claim 78, wherein at least 15 assay samples are analyzed.
80. (Original) The method of claim 78, wherein at least 20 assay samples are analyzed.
81. (Original) The method of claim 78, wherein at least 25 assay samples are analyzed.
82. (Original) The method of claim 78, wherein at least 30 assay samples are analyzed.
83. (Original) The method of claim 78, wherein at least 40 assay samples are analyzed.
84. (Original) The method of claim 78, wherein at least 50 assay samples are analyzed.
85. (Original) The method of claim 78, wherein at least 75 assay samples are analyzed.
86. (Original) The method of claim 78, wherein at least 100 assay samples are analyzed.

87. (Original) The method of claim 78, wherein at least 500 assay samples are analyzed.
88. (Original) The method of claim 78, wherein at least 1000 assay samples are analyzed.
89. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein the source of the sample is selected from the group consisting of stool, blood and lymph nodes.
90. (Canceled)
91. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein the amplifying step employs a polymerase chain reaction.
92. (Currently Amended) The method of claim ~~90~~ 65, 66, 67, 110, 111, or 112, wherein the first probe detects a wild-type nucleic acid better than a mutant nucleic acid.
93. (Currently Amended) The method of claim ~~90~~ 65, 66, 67, 110, 111, or 112, wherein the first probe detects a mutant nucleic acid better than a wild-type nucleic acid.
94. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, further comprising an asymmetric amplification step comprising adding a single internal primer and performing amplification.
95. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein the target nucleic acid comprises one or more mutations selected from the group consisting of base substitutions, chromosomal translocations, gene amplifications and alternatively spliced RNAs.
96. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein said method detects allelic discrimination, allelic imbalance, amplicons that are amplified during neoplastic development or rare exons.

97. (Currently Amended) The method of claim 65, 66, ~~or 67~~, 110, 111, or 112, wherein said method detects a change in gene expression or a change in relative expression of a wild-type nucleic acid or a mutant nucleic acid.

98. (Currently Amended) The method of claim 90 110, 111, or 112, wherein the ~~first detectable probe is a~~ first molecular beacon probe ~~comprising~~ comprises a first stem and a first loop structure ~~and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end~~, wherein the first loop comprises about 16 base pairs and has a ~~T<sub>m</sub>~~ T<sub>m</sub> of about 50-51°C, and wherein the first stem comprises about 4 base pairs having a sequence 5'-CACG-3'.

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99. (Currently Amended) The method of claim 98, wherein the first loop consists of 16 base pairs and has a ~~T<sub>m</sub>~~ T<sub>m</sub> of 50-51°C, and wherein the first stem consists of 4 base pairs having a sequence 5'-CACG-3'.

100. (Currently Amended) The method of claim 89 65, 66, or 68, wherein the ~~second detectable probe is a second~~ molecular beacon probe ~~comprising~~ comprises a second stem and a second loop structure ~~and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end~~, wherein the second loop comprises about 19-20 base pairs and has a ~~T<sub>m</sub>~~ T<sub>m</sub> of about 54-56°C, and wherein the second stem comprises about 4 base pairs having a sequence 5'-CACG-3'.

101. (Currently Amended) The method of claim 100, wherein the second loop consists of 19-20 base pairs and has a ~~T<sub>m</sub>~~ T<sub>m</sub> of 54-56°C, and wherein the second stem consists of 4 base pairs having a sequence 5'-CACG-3'.

102. (Currently Amended) The method of claim ~~65, 66, or 67~~, 111, or 112; wherein said

dividing step comprises diluting said sample.

103. (Currently Amended) The method of claim 65, 66, ~~or~~ 67, 110, 111, or 112, wherein the target nucleic acid comprises one or more somatic mutation.

104-108. (Withdrawn)

109. (New) The method of claim 65 or 110 wherein the separating step comprises diluting said sample.

110. (New) A method for detecting a target nucleic acid, the method comprising the steps of:

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separating a sample in which a target nucleic acid is present in an amount less than about 20% relative to non-target nucleic acid in said sample, to form a plurality of assay samples;

amplifying said target nucleic acid in said assay samples;

hybridizing the amplification products to a first molecular beacon probe which hybridizes to the target nucleic acid and to a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the second molecular beacon probe further comprises a second stem which comprises about 4 base pairs having a sequence 5'-CACG-3', and a second loop structure which comprises about 19-20 base pairs and has a  $T_m$  of about 54-56°C; and

detecting the first and the second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

111. (New) A method for detecting a target nucleic acid, the method comprising the steps of:

providing a sample comprising X% of a target nucleic acid, wherein X is less than 100;

dividing the sample to produce a plurality of assay samples;  
wherein the ratio of target to non-target nucleic acid in at least one of the samples is greater than X%;

amplifying the target nucleic acid to form an amplification product;

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hybridizing the amplification products to a first molecular beacon probe which hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the second molecular beacon probe further comprises a second stem comprising about 4 base pairs having a sequence 5'-CACG-3', and a second loop structure comprising about 19-20 base pairs and a  $T_m$  of about 54-56°C; and

detecting the first and second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

112. (New) A method for detecting a target nucleic acid in a population of non-target nucleic acid contained in a sample, the method comprising:

dividing a heterogenous sample comprising target nucleic acid and non-target nucleic acid to form a plurality of assay samples, wherein the concentration of non-target nucleic acid is at least 5 fold that of target nucleic acid in the heterogeneous sample, and



wherein at least one of the assay samples comprises a single molecule of the target nucleic acid;

amplifying the single molecule of target nucleic acid to form an amplification product;

hybridizing the amplification products to a first molecular beacon probe which hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the second molecular beacon probe further comprises a second stem comprising about 4 base pairs having a sequence 5'-CACG-3', and a second loop structure comprising about 19-20 base pairs and a  $T_m$  of about 54-56°C; and

detecting the first and second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

113. (New) The method of claim 109 wherein the sample is diluted so that between about 0.1 and about 0.9 of the assay samples yield an amplification product when amplified.

114. (New) The method of claim 113 wherein the sample is diluted so that between about 0.1 and about 0.6 of the assay samples yield an amplification product when amplified.

115. (New) The method of claim 65, 66, or 67, wherein the first loop consists of 16 base pairs and has a  $T_m$  of 50-51°C, and wherein the first stem consists of 4 base pairs having a sequence 5'-CACG-3'.

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116. (New) The method of claim 110, 111, or 112, wherein the second loop consists of 19-20 base pairs and has a  $T_m$  of 54-56°C, and wherein the second stem consists of 4 base pairs having a sequence 5'-CACG-3'.

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